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(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Determination of Peptide Motifs on MHC Molecules

(72) Rötzschke, Olaf - U.S.A. ;
Falk, Kirsten - U.S.A. ;
Stevanovic, Stefan - Germany (Federal Republic of) ;
Jung, Günther - Germany (Federal Republic of) ;
Rammensee, Hans-Georg - Germany (Federal Republic of) ;

(71) Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. - Germany (Federal Republic of) ;

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Abstract

A process is disclosed for determining allele-specific peptide motifs on molecules of the major histocompatibility complex (MHC) of classes I and II as well as the peptide motifs obtained by this process. Also disclosed is the use of said peptide motifs for preparing a diagnostic or therapeutical agent

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Determination of peptide motifs on MHC molecules

Description

The present invention concerns a method for the determination of peptide motifs or epitopes on molecules of the major histocompatibility complex (MHC) as well as the peptide motifs which are determined by this means and their use for the production of a diagnostic or therapeutic agent.

The cytotoxic T lymphocytes (CTL) recognize antigenic peptide epitopes in association with MHC-coded molecules. This phenomenon is called MHC restriction (1-5). Crystallography of human MHC class I molecules, HLA-2 and Aw68, revealed a groove which is formed by the α1 and α2 domains of the heavy chains (3,6). It is presumed that this groove is the binding site for antigenic peptide epitopes since both crystals contained structures of peptide size which were not compatible with MHC sequences and were located at this groove (6).

It is assumed that these peptides are derived from intracellular proteins and are presented at the cell surface in order to allow the cytotoxic T lymphocytes to check the cells for abnormal properties. MHC-associated peptides which represent T cell epitopes have already been extracted from normal or virally infected cells (2,4,5,7,8). Antigens which are recognized by the MHC class II-restricted T cells can also be mimicked in a corresponding manner by artificial peptides (9) and MHC-associated antigenic peptides were eluted by MHC class II molecules (10). Due to their position at the centre

of trimolecular complexes which consist of T cell receptor, peptide and MHC molecule (11), the T cell epitopes are a central point of the specific immune system and thus there is a great need to understand the rules governing their occurrence and for a method of determination (12-15).

The object according to the invention is achieved by a method for the determination of allele-specific peptide motifs on molecules of the major histocompatibility complex (MHC) of classes I or II wherein

(a) a cell extract is produced by lysing cells which contain MHC molecules,

(b) MHC molecules with the peptide mixtures which are located thereon are separated from the cell extract by immunoprecipitation,

(c) the peptide mixtures are separated from MHC molecules and other protein components,

(d) individual peptides or/and a mixture thereof are sequenced and

(e) the allele-specific peptide motif is derived from the information obtained, in particular from the sequencing of a mixture or from the sequencing of a number of individual peptides

which is characterized in that peptide motifs are determined on molecules which are selected from the group comprising HLA-A1, HLA-A3, HLA-A11, HLA-A24, HLA-A31, HAL-A33, HLA-B7, HLA-B8, HLA-B*2702, HLA-B*3501, HLA-B*3503, HLA-B37, HLA-B38, HLA-B*3901, HLA-B*3902, HLA-B*5101, HLA-B*5102, HLA-B*5103, HLA-B*5201, HLA-B53, HLA-B60, HLA-B61, HLA-B62, HLA-B78, HLA-Cw*0301, HLA-Cw*0401, HLA-Cw*0602, HLA-Cw*0702, HLA-Cw4, HLA-Cw6, HLA-Cw7, HLA-DRB1*0101, DRB1*-1201, HLA-DR4w14, HLA-DR17, HLA-DRw52, HLA-DPw2, HLA-DPB1*0401, HLA-DQB1*0301, HLA-DQw1, HLA-DR1, HLA-DR3 and HLA-DR5.

Peptide motifs are determined by the method according to the invention which comprise the rules by which MHC molecules select and present peptides.

The method according to the invention can be carried out with MHC molecules of class I as well as with MHC molecules of class II, whereby MHC molecules of class I are preferred. The peptide motifs HLA-A, HLA-B and HLA-C are ligands for MHC-molecules of class I. The peptide motif HLA-DR, HLA-DQ and HLA-DP are ligands for MHC molecules of class II.

When MHC molecules are immunoprecipitated by the method according to the invention, it is advantageous to use antibodies which are specific for the MHC molecules which are desired in each case. For the determination of H-2K^d or H-2D^b molecules, K^d-specific antibodies (25) or D^b-specific antibodies (26) are for example used. Monoclonal antibodies are preferably used, it is however, also possible to use an appropriately purified polyclonal antiserum. Antibodies which can be used according to the invention can be produced de novo by means of standard techniques which are well known to a person skilled in the art. Examples of antibodies which can be used in the invention include all antibodies against HLA antigens, which are mentioned in the "Catalogue of Cell Lines and Hybridomas" of the ATCC (American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852) but are not limited to these. Preferred examples (in the ATCC nomenclature), include HB82, 117, 166, 54, 122, 164, 95, 120, 116, 118, 94, 152, 178, 56, 115, 157, 119, 59, 105, 165, 144, 180, 103, 110, 109, 151 and 104. All antibodies against mouse H-2 antigens mentioned in the catalogue can also be used in the invention. The immunoprecipitation is

particularly preferably carried out by solid phase-bound antibodies. Solid phase-bound antibodies can be produced in a manner well known to a person skilled in the art for example by coupling the antibody to cyanogen bromide-activated Sepharose 4B (Pharmacia LKB). Other examples of solid phases to which antibodies can be bound for the use according to the invention include agarose, cellulose, Sephadex, protein-A-Sepharose and protein-G-Sepharose but are not limited to these. The preferred method of immunoprecipitation is adsorption chromatography by means of antibodies which are coupled to beads which are manufactured from cyanogen bromide-activated Sepharose 4B (see example 1).

The separation of the peptide mixtures to be determined from MHC molecules and other protein components is advantageously carried out by a chromatographic method, preferably by reverse phase HPLC. In this connection it has proven to be advantageous to carry out the separation in a trifluoroacetic acid/H_2O-trifluoroacetic acid/acetonitrile gradient. Other methods which can be used according to the invention to separate peptide mixtures from MHC molecules include ion exchange, gel filtration, electrofocussing, high performance capillary electrophoresis (HPCE) and gel electrophoresis but are not limited to these. Another means for carrying out the separation is ultrafiltration in which a membrane with a permeability of 3000 or 5000 or 10000 Da is used. The separation is preferably carried out by means of HPLC.

In the chromatographic separation of the peptide mixtures it is possible in some cases to isolate a single peptide species. Consequently, step (d) of the method according to the invention comprises either the sequencing of a peptide mixture by which means a

consensus sequence can be determined for the peptide motifs which are located on the respective MHC molecule or/and sequencing a defined peptide.

Normal cells, tumour cells as well as cells infected by viruses or other pathogens and in vitro cultured cells of humans or animals can be used as the starting material for the determination of peptide motifs. Normal cells which can be used in the invention include but are not limited to fresh cells such as e.g. peripheral blood lymphocytes, cells of the spleen, lung, thymus or cells of another tissue which expresses MHC molecules. Tumour cell lines used in the invention include the tumour cells EL4 and P815 but are also not limited to these. Virally infected cells which can be used in the invention include but are not limited to JY cells which are human B cells transformed by the Epstein-Barr virus. The peptide motifs determined by the method according to the invention correspond to the following basic principle:

a) They have an allele-specific peptide length of 8, 9, 10, or 11 amino acids in MHC class I molecules as well as of 8 to 15 amino acids in MHC class II molecules,

b) they have two anchor positions (the term "anchor position" is used when a position shows a strong signal for a single amino acid residue or when a position is occupied by a few amino acid residues with very closely related side chains) of which one anchor position is always located at the C-terminal end and is frequently aliphatic and

c) the peptides are naturally presented on MHC molecules of normal, virally infected or otherwise infected cells or cells transfected with genes or coated with antigen.

The sequencing of the self-peptide mixtures from the MHC class I molecules H2K^d, H2K^b, H2D^b and HLA-A2 shows a different allele-specific peptide motif in each case which is presented by each of the class I molecules. The peptides presented by K^d, D^b and A2 are nonamers whereas the K^b-presented peptides are octamers and the corresponding peptide motifs contain two anchor positions which are occupied by a single amino acid residue or by a small number of amino acid residues with closely related side chains. These anchor positions are not located at the same site in the various motifs, they can for instance be at position 5 and 9 (D^b) or 2 and 8 (K^d, A2) or 5 and 8 (K^b). The C-terminal anchor residues of all motifs are hydrophobic amino acids. The amino acid residues which are not located at anchor positions can be quite variable; some however, are chiefly occupied by particular amino acids, for example Pro is often found at position 4 of the K^d motif, Tyr at position 3 of the K^b motif and hydrophobic residues are predominant at positions 3 of the D^b motif and 6 of the A2 motif. A proline anchor residue was at position 2 of H-2L^d.

The results obtained by the method according to the invention correspond very well with the structure of the groove in MHC class I molecules found by crystallography (3,6). Different MHC class I alleles differ at this groove by the presence of different pockets which is presumably due to the fact that the pockets can accomodate different amino acids in each case. Thus the

allele-specific pockets in the MHC crystals and the side chains of the allele-specific anchor residues presumably represent complementary structures.

The present invention in addition concerns the use of the peptide motifs according to the invention in a process for the production of a diagnostic or therapeutic agent. A possible area of application for the peptide motifs is the diagnostic detection of MHC molecules. Since the MHC molecules are characterized by their individual specific binding of peptides, a binding test can be carried out by means of peptides with a marker group in which for example a biotin or a fluorescent group is coupled to the peptide as the marker group. Other labels known to a person skilled in the art can also be used in the invention. These labels include, without being limited thereto, radioactive markers such as e.g. ^{131}I, or ^{125}I bound to the tyrosine residues of peptides or ^{3}H or ^{14}C (both of which are incorporated into the peptides during their synthesis). Binding of the labels to the peptides can be achieved according to methods well known to a person skilled in the art. The labelling is preferably carried out at non-anchor positions. The correlations between the occurrence of autoimmune diseases and the expression of MHC molecules with disease-specific peptide motifs which are found in this manner can be utilized diagnostically. Examples of in vitro diagnostic uses of the peptide sequences according to the invention include, without being limited thereto, measurement of the binding specificity of MHC molecules, correlation of the binding specificity of MHC molecules with diseases, and determination of the sequence of T cell epitopes of unknown origin by incubating suitable cells which express the MHC molecules of interest with HPLC

fractions of a peptide library (mixture of peptides which fit into the motif being examined) and determining the peptides recognized by the T cell, followed by a chromatographic comparison of the natural T cell epitope with the synthetic peptide recognized as the T cell epitope (Nature 348: 252-254 (1990)).

The invention in addition concerns the use of the peptide motifs according to the invention in a process for the production of a therapeutic agent for the therapy of disturbances of the immune system or of tumour diseases. In particular the peptide motifs according to the invention can be used for intervention in autoimmune diseases (prophylaxis and therapy), for example by blocking certain MHC molecules as well as by inducing the peptide-specific non-reactivity of T cells. In addition an intervention in transplant rejections and graft-versus-host reactions is also possible in an analogous manner. In addition the peptides according to the invention can be used in vitro and in vivo for the induction or amplification or proliferation of T cells directed against tumour cells in particular for vaccination against tumour diseases and for the therapy of existing tumour diseases in which in particular the so-called graft-versus-leukemia effect (Sullivan et al., N. Engl. J. Med. 320: 828-834) can be utilized. The peptides according to the invention can also be used to amplify T cell responses towards infectious or malignant diseases by employing MHC-binding peptides in vivo which are specific for the infectious agent or for tumours. Alternatively, T cells can be obtained from patients, their number increased in vitro by using peptides and suitable growth conditions, including cytokines such as e.g. interleukin 2, interleukin 4 or interleukin 6, and subsequently returned to the patient. In addition the

peptides according to the invention can be used to treat all tumours which express antigens which can be attacked by T cells including, but not being limited to, melanomas, breast cancer, tumours of viral origin such as e.g. Burkitt's lymphoma and those tumours which are caused by human papilloma virus such as cervical carcinoma and other anogenital tumours. Peptides which are derived from T cell receptor molecules or antibody molecules can also be utilized for the targetted manipulation of immunoregulatory mechanisms, in particular for the control of autoimmune diseases and transplant rejections as well as graft-versus-host reactions. In vivo uses of the proteins according to the invention for prevention include without being limited to their use as peptide vaccines against infectious or malignant diseases and use of the information compiled in this invention with regard to suitable T cell epitopes for incorporation into all other types of vaccines including recombinant vaccines (including viruses such as vaccinia or bacteria such as salmonella or mycobacteria) and proteins which have been produced by using recombinant bacteria (e.g. E. coli) or other cells, including yeast, insect, murine or human cells.

The dosage or concentrations of the peptides according to the invention can be routinely determined by a person skilled in the art. These can be expected in vivo to be in a range of 10 µg to 1 g. In vitro concentrations can be expected to be in a range of 1 femtomole to 1 micromole. The in vivo administration includes, but is not limited to, a subcutaneous, intramuscular, intraveneous, intradermal and oral route.

In the therapeutic application, a peptide which corresponds to a peptide motif according to the

invention is preferably covalently linked at the N- or/and C-terminus to lipophilic or amphiphilic groups, in particular lipophilic peptide helices. An example of such a group is tripalmitoyl-S-glycerylcysteinyl-serylserine.

It is intended to elucidate the invention further by the following examples in conjunction with Figure 1.

Fig. 1a shows a HPLC profile of material which was separated from P815 lysate using anti-K^d antibodies,

Fig. 1b shows an enlarged section from the chromatogram of 1a (fractions 15 - 35),

Fig. 1c shows a rechromatography of the self peptide indicated by the arrow in 1b.

Fig. 2 shows MHC molecules and their ligands.

Example 1

10 to 20x10⁹ P815 tumour cells (H-2K^d) were pelleted and stirred for 30 minutes at 4°C with 250 ml 0.5 % Nonidet P40 in phosphate-buffered saline solution (PBS) containing 0.1 mmol/l phenylmethylsulfonyl fluoride (PMSF). The supernatant was centrifuged at 4°C for 5 minutes at 250 g and 30 minutes at 150000 g and then passed through an arrangement for adsorption chromatography. The arrangement for adsorption chromatography consisted of three columns each with a bed volume of about 1 ml. The column material was composed of antibody-coupled or glycine-coupled beads which were produced from cyanogen bromide-activated Sepharose 4B (Pharmacia LKB) according to the protocol of the manufacturer. In each case 5 mg of K^d-specific antibody 20-8-4S (IgG 2a, kappa; 25) or D^b-specific

antibodies B22-249 (IgG 2a, kappa; 26) coupled to 1 ml of the beads was used as the antibody. The supernatant of the cell extract was firstly passed through a column with glycine-coupled beads then through a corresponding column with anti-K^d beads and then over anti-D^b beads for a sham precipitation.

The beads were removed from all three columns and whirlimixed with 0.1 % trifluoroacetic acid for 15 minutes (7). The supernatants were dried by vacuum centrifugation and separated by reverse phase HPLC using a Superpac Pep S column (C2/C18; 5 µm particles, 4.0 x 250 mm, Pharmacia LKB) and a Pharmacia LKB apparatus (4). Eluting agent: solution A 0.1 % trifluoroacetic acid in H_2O (v/v), solution B 0.1 % trifluoroacetic acid in acetonitrile.

The following gradient was used for the chromatographic separations shown in Figures 1a and b:
0 to 5 minutes, 100 % A
5 to 40 minutes linear increase to 60 % B,
40 to 45 minutes 60 % B,
45 to 50 minutes decrease to 0 % B,
flow rate: 1 ml/minute, fraction size 1 ml.
The individual fractions were collected and dried by vacuum centrifugation.

Figure 1 shows the HPLC separation of immunoprecipitated K^d molecules treated with trifluoroacetic acid. Figure 1a shows a HPLC profile of TFA-treated material which was precipitated from P815 lysate with anti-K^d (continuous line) or with anti-D^b (dashed line). Heterogeneous material is eluted between fractions 20 and 28 in small amounts which represents the desired

allele-specific peptide mixtures.

Fractions 20 to 28 were collected from the K^d preparation as well as from the sham precipitate Both preparations were automatically sequenced using the Edman degradation method (Edman et al., Eur. J. Biochem. 1: 80-91 (1967)). The Edman degradation was carried out in a protein sequencer 477A, equipped with an on-line PTH amino acid analyzer 120A (Applied Biosystems, Foster City, CA, 94404, USA). Glass fibre filters were coated with 1 mg BioPrene Plus and were not pre-cyclised. The sequencing was carried out using the standard programme BEGIN-1 and NORMAL-1 (Applied Biosystems). Cysteine was not modified and also could therefore not be detected.

The Edman method includes a sequential derivatization and amino acid removal starting at the N-terminus, each of which is identified by chromatography. Since it is unusual to sequence complex mixtures of peptides, the data obtained directly from the sequencing instrument are presented. Tables 1a and b show the results from two sequencing experiments for K^d-eluted peptides. Table 1c shows the sequencing result for a sham elution with D^b-specific antibodies on P815 lysates. The K^d-eluted peptides have a clear amino acid pattern for each position from 1 to 9 whereas the sham-eluted material has a uniform amino acid pattern throughout with a decrease in the absolute amount of each residue in each cycle. In the K^d-eluted peptides, only those residues which showed a more than 50 % increase in their absolute amount compared to the previous cycle or the cycle before last were regarded as significant and are underlined. The first position is difficult to judge since it has no previous cycle and moreover all free amino acids present in the HPLC pool are detected at

this position. The only residue at the second position whose frequency is clearly increased in comparison to the previous cycle is tyrosine (e.g. Table 1a from 60.9 pmol to 875.6 pmol). The only other residue which shows a (small) increase is phenylalanine which has a similar side chain to Tyr. This confirms the assumption which results from a comparison of the natural K^d-restricted influenza epitope (with the sequence TYQRTRALV) with other K^d-restricted peptides with regard to the tyrosine residue at position 2. In contrast there are no definite amino acid residues which are characteristic for the following positions 3 to 8. Up to 14 different residues are found at the individual positions. Ile and Leu are found at position 9. There is no increase in signal at position 10 which indicates that most of the K^d-bound self peptides are not longer than 9 residues. The natural K^d-restricted influenza peptide is thus a nonapeptide (4). The consensus sequence pattern which is derived from these results is shown in Table 1c. The most striking features are Tyr at position 2 and Ile or Leu at 9 whereas a large number of residues are found at all other positions. A comparison of this motif with peptide sequences which contain K^d-restricted epitopes shows that most of them match well with the K^d-restricted consensus monomer motif (Table 1d).

The peak in fraction 29 of Figure 1b marked with an arrow and the corresponding fraction of the sham precipitation were chromatographed again using a higher resolution in which the fraction volume was 0.5 ml (Fig. 1c). The sharp specific peak represents a peptide with the amino acid sequence SYFPEITHI which was determined by direct sequencing. Coelution on HPLC (Fig. 1c) confirmed that this natural cell peptide is identical to

the synthetic SYFPEITHI peptide. The sequence matches the consensus motif from the pool of fractions 20 to 28 (Fig. 1a, b) which thus confirms the presence of a specific K^d-restricted peptide motif (Table 1d).

Table 1

Sequencing of the self peptide mixture which was eluted from immunoprecipitated K^a molecules.

Cycle	A Ala	R Arg	N Asn	D Asp	E Glu	Q Gln	G Gly	H His	I Ile	L Leu	K Lys	M Met	F Phe	P Pro	S Ser	T Thr	Y Tyr	V Val
(a) Experiment 1 — Amino acid residues (in pmol)																		
1	172.0	46.1	44.9	13.6	73.5	317.0	171.6	3.2	70.1	66.5	231.2	28.0	35.3	56.7	145.2	73.3	60.9	130.9
2	25.6	14.1	10.1	7.7	10.7	71.9	71.9	1.2	20.4	22.6	13.9	11.1	97.7	14.8	14.6	9.3	875.6	18.8
3	80.7	26.7	51.5	10.0	23.1	86.8	62.5	2.9	183.2	500.7	71.6	25.6	41.5	13.5	24.0	22.0	66.1	150.2
4	159.5	14.2	31.9	17.9	53.3	44.8	85.2	6.7	32.1	36.6	29.5	9.2	5.8	276.9	26.2	19.9	14.7	41.5
5	139.0	30.1	42.2	22.9	15.1	44.1	154.5	1.8	59.3	86.6	10.2	50.8	2.6	87.8	64.2	47.6	8.8	104.2
6	116.5	29.2	42.6	13.0	10.6	38.3	139.1	0.5	90.1	99.9	194.5	69.7	27.5	38.6	15.1	26.5	35.9	106.8
7	51.5	79.7	125.1	25.8	47.0	71.7	65.8	7.9	12.8	23.4	37.8	11.2	5.1	16.9	30.3	148.4	11.2	36.1
8	44.2	29.0	48.9	22.4	75.8	58.0	59.0	18.3	10.1	30.4	41.5	10.5	19.3	10.8	28.8	46.0	47.9	63.2
9	13.0	8.3	20.1	10.7	14.4	10.4	20.5	3.5	129.4	155.2	3.9	4.9	5.0	7.2	7.0	10.1	9.4	35.4
10	6.5	4.4	7.8	6.1	4.2	5.6	14.6	1.3	32.1	58.3	3.1	1.8	3.1	4.7	4.2	5.2	4.3	8.8
(b) Experiment 2																		
1	54.5	0.4	5.0	3.5	5.0	5.8	62.5	1.8	11.2	13.2	35.3	5.8	11.5	35.3	57.8	26.0	15.1	29.2
2	14.1	0.2	1.2	1.0	2.2	3.6	20.0	0.5	3.4	5.7	3.4	1.6	19.6	8.6	8.5	5.1	187.7	5.5
3	22.4	4.4	10.3	2.5	7.1	15.9	20.2	0.8	41.0	77.2	12.7	7.5	23.0	6.6	6.7	5.3	16.9	27.7
4	40.2	1.4	11.7	5.5	23.8	8.1	34.3	2.3	7.5	10.4	4.9	3.7	2.1	60.0	6.9	5.7	3.8	12.1
5	35.2	1.7	11.7	8.9	9.1	7.2	41.5	0.7	12.3	18.1	1.4	17.6	0.9	20.7	16.1	11.6	1.7	25.6
6	33.3	5.1	7.0	5.0	6.4	6.5	55.9	1.8	22.4	31.9	31.4	12.9	4.5	9.4	4.2	3.5	5.5	27.0
7	11.2	1.1	22.1	11.8	11.2	15.7	16.0	2.7	5.7	7.0	5.0	2.9	1.1	1.5	12.4	47.3	2.0	8.0
8	10.7	3.4	7.8	7.3	16.5	9.7	19.5	4.3	2.5	8.7	5.0	2.4	4.2	0.8	7.6	10.7	8.2	16.8
9	4.1	2.6	4.0	4.2	4.8	1.9	10.6	0.4	37.0	26.6	0.0	1.3	1.5	0.5	2.3	3.1	1.9	7.7
10	2.5	1.0	1.3	3.1	2.7	1.0	7.5	0.2	13.0	13.5	0.0	1.0	1.3	1.5	1.6	1.4	1.2	3.4
(c) Sequencing of the sham precipitated material																		
1	63.5	5.6	3.6	3.9	8.3	11.3	51.5	2.3	12.2	16.5	8.4	3.5	10.8	47.0	35.2	27.3	12.7	24.4
2	24.8	2.5	3.1	3.6	7.9	6.2	33.8	1.3	6.9	17.1	4.5	1.4	5.8	18.4	7.4	6.4	6.9	13.8
3	15.2	0.9	2.5	3.0	6.9	3.6	26.6	1.2	4.1	11.0	2.7	1.2	4.2	16.1	2.7	4.0	4.3	8.6
4	11.5	1.0	2.2	3.2	5.7	2.6	19.5	0.8	3.9	7.3	2.8	1.1	2.7	10.7	1.6	2.4	3.1	6.4
5	10.5	1.4	2.1	3.1	5.0	2.6	15.7	1.0	3.1	6.2	2.3	0.7	2.2	7.9	0.9	1.7	2.6	5.2
6	8.8	1.1	1.6	3.1	4.1	2.0	12.6	1.1	2.2	4.6	1.9	0.6	1.9	6.5	1.1	1.4	1.9	3.9
7	6.8	1.0	1.6	2.4	3.5	1.8	9.8	0.5	1.8	3.4	2.1	0.4	1.7	4.3	1.6	1.5	1.7	2.7
8	0.0	0.3	0.0	2.1	0.2	0.0	0.0	0.6	1.1	2.8	1.7	0.3	1.1	3.6	0.0	2.2	0.2	2.6
9	0.1	0.6	0.0	1.8	0.0	0.0	0.7	0.2	1.6	2.5	1.7	0.5	1.1	3.3	1.3	1.7	0.1	2.1
10	0.2	0.3	0.0	1.7	0.1	0.5	0.0	0.2	1.0	2.5	1.4	0.1	1.3	2.7	0.8	1.7	0.1	2.1

2147863

Table 1d

The Kd-restricted peptide motif

	Position								
	1	2	3	4	5	6	7	8	9
Dominant anchor residues		Y							I L
strong			N I L	P	M	K F	T S		
weak	X A R S V T	F	A H V R S F E Q K M T	A E S D H N	V N D I L S T G	H I M Y V R L	E D E Q E	H E K V V F R	

Known epitopes*	Protein source	Literature reference
T Y Q R T R A L V	influenza PR8 NP 147-154	4,29
S Y F P E I T H I	self peptide P815	
I Y A T V A G S L	influenza JAP HA 523-549	30,31
V Y Q I L A I Y A	influenza JAP HA 523-549	30,31
I Y S T V A S S L	influenza PR8 HA 518-528	32
L Y Q N V G T Y V	influenza JAP HA 202-221	30,31
R Y L E N G K E T L	HLA-A24 170-18233	33
R Y L K N G K E T L	HLA-Cw3 170-186	34
K Y Q A V T T T L	P815 tumour antigen	35
S Y I P S A E K I	Plasmodium berghei CSP 249-260	36
S Y V P S A E Q I	Plasmodium yoeli CSP 276-288	37

* Peptides which are known to contain K^d-restricted T cell epitopes were aligned with respect to their Tyr residues. Peptides which are known to be naturally processed are underlined.

Example 2

Elution of peptides from K^b and D^b molecules

Detergent lysates from EL4 tumour cells ($H\text{-}2^b$) were immunoprecipitated with K^b-specific and D^b-specific antibodies as described in example 1. B22-249 (see example 1) was used as the D^b antibody and K9-178 (IgG 2a, K, 27) was used as the K^b antibody. The peptides dissociated from MHC molecules were separated by reverse phase HPLC. K^b material as well as D^b material was eluted with profiles which corresponded approximately to the K^d material from example 1 but, however, there were certain differences in the heterogeneous material which eluted between fractions 20 and 28.

D^b-restricted peptide motif

The combined fractions 20 to 28 from the D^b preparation were sequenced (Table 2a, b). Positions 2 to 4 contained several residues. In contrast cycle 5 gave a strong signal for Asn. The predominant residue at position 5 of the D^b-eluted self peptides is thus Asn. The weak signal for Asp is caused by hydrolysis of Asn to Asp under the sequencing conditions. Positions 6 to 8 contain 5 to 14 different detectable residues. Position 9 contained a strong signal for Met, a moderate signal for Ile and a weak signal for Leu (all hydrophobic). (The significance of Met or Ile in a D^b-restricted epitope has already been reported, see 17). At position 10 there was no signal which indicates that D^b-presented self peptides are non-peptides. The consensus motif determined from these results is shown in Table 2c. A comparison of this

motif with the natural D^b-restricted peptide and with other peptides which contain D^b-restricted epitopes shows that Asn at position 5 may be an invariable anchor residue of the D^b-restricted peptide motif. The other residues of the D^b-restricted epitopes differ considerably with the exception of position 9 (with Met, Ile or Leu) which looks like a second anchor position.

Table 2

Sequencing of the self peptide mixture which was eluted from D^b molecules

Cycle	A Ala	R Arg	N Asn	D Asp	E Glu	Q Gln	G Gly	H His	I Ile	L Leu	K Lys	M Met	F Phe	P Pro	S Ser	T Thr	Y Tyr	V Val
(a) Experiment 1							Amino acid residues		(in pmol)									
1	251.2	10.2	21.6	1.3	0.1	16.3	99.1	2.3	22.0	21.2	20.3	7.2	33.0	27.5	124.6	43.9	26.9	70.1
2	202.1	7.2	5.4	6.8	7.4	24.7	116.2	0.9	5.4	9.9	6.5	154.1	4.3	8.2	52.7	15.0	5.5	16.0
3	29.9	5.9	5.3	0.0	3.8	5.5	185.1	1.1	106.3	65.8	0.0	8.3	3.8	88.1	8.3	4.7	5.2	23.2
4	18.3	0.1	4.2	4.6	32.4	21.0	49.3	0.8	32.7	21.5	12.4	3.6	2.3	26.8	9.9	18.6	5.0	165.2
5	6.8	2.1	271.4	26.0	8.2	4.3	43.0	0.6	4.7	6.2	2.5	1.3	0.9	11.7	4.5	5.0	1.7	7.6
6	42.1	5.9	29.6	7.1	8.4	7.8	32.6	1.3	18.0	148.4	0.8	1.9	11.3	22.5	7.8	11.8	4.1	23.6
7	21.5	20.4	10.2	24.5	30.4	13.7	22.0	0.7	9.9	16.2	2.4	2.1	3.6	16.4	6.7	54.3	5.1	35.0
8	14.6	10.1	11.3	9.8	23.2	10.3	18.2	0.3	3.0	10.1	4.4	1.3	5.0	9.5	26.5	24.9	12.5	20.7
9	7.5	3.2	7.9	3.2	3.1	1.6	11.2	0.5	8.5	13.7	0.5	7.7	3.0	2.5	2.0	3.3	3.6	3.5
10	2.6	1.1	2.5	2.4	1.9	1.2	12.5	0.3	4.2	8.5	0.4	2.7	1.8	2.1	1.6	1.7	1.9	1.3
(b) Experiment 2																		
1	413.4	45.8	29.7	15.9	14.5	19.6	132.4	4.7	41.5	40.8	48.9	17.2	50.8	26.1	307.7	94.0	47.4	110.1
2	227.4	14.4	7.6	9.3	11.1	25.2	133.8	2.1	8.2	14.5	13.3	169.9	5.6	4.9	71.0	21.6	11.3	22.6
3	39.6	3.3	6.0	6.3	6.0	5.3	172.2	1.2	89.5	56.0	1.6	14.7	4.5	75.4	12.1	5.0	7.6	19.2
4	29.3	16.6	6.7	10.6	34.8	23.0	57.3	0.8	36.3	21.7	17.0	8.1	4.2	33.5	12.5	23.9	7.4	198.9
5	19.9	5.3	154.7	22.2	8.7	4.1	31.1	0.9	4.6	7.0	4.3	2.4	1.7	11.8	5.3	5.0	2.0	13.8
6	42.3	8.4	30.8	15.7	14.6	8.3	28.7	2.3	18.6	124.1	8.2	5.3	11.2	22.1	7.9	10.7	3.6	29.2
7	22.0	24.5	1	33.5	29.2	10.5	17.7	1.6	11.3	14.8	3.3	3.7	3.6	14.3	7.5	47.3	6.9	35.5
8	15.8	10.9	10.2	20.9	25.6	8.0	12.6	3.2	3.3	13.6	4.3	2.8	5.1	8.7	20.8	19.3	12.9	23.6
9	8.7	4.3	6.1	13.0	12.1	2.6	8.7	0.3	19.8	26.2	1.2	30.8	3.9	4.4	4.8	5.6	7.2	9.2
10	5.4	3.1	3.9	12.2	8.1	2.0	8.2	0.0	10.1	13.9	0.7	11.6	3.2	3.4	3.0	3.0	7.3	5.9

2147363

Table 2c

The D^b-restricted peptide motif

	Position								
	1	2	3	4	5	6	7	8	9
Dominant anchor residues					N				M
strong		M	I	K		L			I
			L	Ξ		F			
			P	Q					
			V	V					
weak	A	A	G	D		A	D	F	L
	N	Q		T		Y	E	H	
	I	D				T	Q	K	
	F					V	V	S	
	P					M	T	Y	
	S					E	Y		
	T					Q			
	V					H			
						I			
						K			
						P			
						S			

Known epitopes

	Protein source	Literature reference
A S N E N M E T M	influenza NP 366-374 154	4,2
S G P S N T P P E I	adenovirus E1A	38
S G V E N P G G Y C L	lymphocytic choriomeningitis virus GP 272-293	39
S A I N N Y . . .	simian virus 40 T 193-211	40

K^b-restricted peptide motif

The combined fractions 20 to 28 from the K^b preparation were sequenced (Table 3a, b). Position 3 contained a strong signal for Tyr and a weak one for Pro. Position 4 showed weak signals for 5 residues. Strong signals for Phe and Tyr make both these residues predominant at position 5. The next two positions contained 5 and 3 signals respectively. Position 8 showed a strong signal for Leu, a moderate one for Met and weaker ones for Ile and Val. Position 9 showed no increase for any residue which is in agreement with the length of the known K^b-restricted natural peptide which is an octamer (5). An analysis of the K^b-restricted consensus motif and comparison with epitopes shows two anchor positions: Tyr or Phe (both with similar aromatic side chains) at position 5 and Leu, Met, Ile or Val (all with similar hydrophobic side chains) at position 8.

Table 3

Sequencing of the self peptide mixture eluted from K^b molecules

Cycle	A Ala	R Arg	N Asn	D Asp	E Glu	Q Gln	G Gly	H His	I Ile	L Leu	K Lys	M Met	F Phe	P Pro	S Ser	T Thr	Y Tyr	V Val
(a) Experiment 1							Amino acid residues (pmol)											
1	978.7	26.3	49.2	55.0	30.0	23.1	514.9	20.9	167.5	167.2	109.8	50.3	116.7	110.2	120.8	365.2	136.0	352.5
2	345.5	3.9	37.3	41.0	23.5	20.3	475.2	8.9	44.5	43.1	72.6	12.6	25.4	51.0	253.1	80.5	50.1	93.5
3	129.0	1.4	14.7	37.0	17.7	9.8	350.0	5.9	8.2	19.0	26.9	4.1	6.0	32.5	56.2	20.0	75.6	25.9
4	52.1	3.5	10.8	45.3	30.0	9.2	246.7	5.0	4.9	7.0	17.7	2.4	1.8	14.6	23.0	13.4	12.0	16.4
5	18.9	13	55	34.7	12.0	3.6	120.2	2.8	1.9	4.7	3.8	1.6	50.5	6.7	8.9	4.6	33.2	4.9
6	16.2	0.8	5.6	32.7	13.0	3.7	77.9	2.4	3.1	3.5	3.9	0.9	4.5	7.3	9.2	16.3	7.3	6.2
7	9.9	0.9	14.9	30.4	9.5	6.6	51.3	0.6	0.0	3.4	9.2	0.5	1.9	4.7	6.1	10.7	3.5	3.4
8	6.0	1.4	5.1	22.7	6.0	3.3	29.2	0.8	1.4	13.5	1.8	7.1	1.0	3.8	4.1	3.1	2.5	3.6
9	4.6	1.5	2.6	19.9	4.5	2.3	21.1	0.9	0.9	6.9	1.0	1.5	1.0	3.0	3.7	7.2	1.9	2.1
10	3.9	0.5	1.9	17.5	3.7	2.1	17.5	1.0	0.5	4.0	0.8	0.5	1.2	2.0	3.5	1.8	2.0	1.5
(b) Experiment 2																		
1	42.4	1.1	5.2	3.0	7.8	17.1	44.6	0.3	11.3	12.6	12.1	3.8	6.2	7.6	44.2	18.1	6.8	26.2
2	24.0	0.2	9.4	2.8	5.1	8.0	42.5	0.5	4.7	6.3	4.0	1.3	3.7	3.5	14.9	10.3	3.1	6.9
3	10.4	0.3	2.1	2.6	3.9	4.0	25.1	0.7	2.8	7.9	2.1	0.9	3.6	9.8	3.0	3.3	16.7	10.0
4	9.6	1.3	2.7	5.7	7.5	4.1	24.5	0.2	1.5	5.0	6.3	0.7	1.5	5.9	3.0	5.9	2.1	4.5
5	5.8	0.8	1.8	2.8	3.3	2.5	14.7	0.5	0.2	3.9	1.7	0.4	10.3	3.5	1.3	2.0	20.0	2.2
6	8.6	0.2	2.3	2.7	6.3	2.7	9.2	0.0	1.0	2.4	1.5	0.4	2.5	3.2	2.7	5.7	3.6	2.4
7	5.0	0.1	8.2	3.3	3.9	4.2	10.4	0.6	0.4	2.3	7.2	0.1	1.2	2.1	1.9	2.8	1.9	1.2
8	4.0	0.1	3.1	2.0	2.6	1.7	6.9	0.2	0.2	13.0	1.6	1.0	0.8	1.1	0.7	1.3	1.1	2.2
9	4.5	0.1	1.1	2.1	3.6	1.9	5.9	1.4	0.0	7.7	0.9	1.0	0.9	1.3	0.3	1.3	0.8	1.8
10	3.9	1.7	0.3	4.5	3.0	1.4	5.4	0.2	0.0	3.9	0.6	0.6	0.6	1.1	0.6	1.1	0.8	1.1

2147363

Table 3c
The Kb-restricted peptide motif

	Position							
	1	2	3	4	5	6	7	8
Dominant anchor residues					F Y			L
strong			Y					M
weak	R I L S A	N	P	R D E K T		T I E S	N Q K	I V

Known epitopes

	Protein source	Literature reference
R G Y V Y Q G L	vesicular stomatitis virus NP 52-59	5
S I I N F E K L	ovalbumin 258-276	41
A P G N Y P A L	sendai virus NP 321-332	42

Example 3
HLA-A2.1-restricted peptide motif

A detergent lysate of human JY cells with the HLA-A2.1 MHC molecule (45) was immunoprecipitated with A2-specific antibodies (BB7.2, IgG2b, literature reference 28). The peptides dissociated from A2 molecules were separated by HPLC. Fractions 20 to 28 were pooled and

sequenced as previously described (Table 4). The second position contained a strong signal for Leu and a moderate one for Met. 6 to 8 residues were found at each of the positions 3 to 5. Position 6 contained Val, Leu, Ile and Thr. The following two positions each showed three signals. Position 9 showed a strong Val signal and a weak Leu signal. Position 10 showed no increase for a residue which indicates that A2-restricted epitopes are nonapeptides. Leu or Met at position 2 and Val or Leu at position 9 appear to be anchor residues. Some of the known peptides with A2-restricted epitopes can be aligned with the motif, whereas this is only partially possible for others (Table 4c). The existence of several variants of A2 molecules may cause this poor correspondence of some peptides with the motif.

Table 4

Sequencing of the self peptide mixture which was eluted from A2.1 molecules

	Amino acid residues (pmol)																	
(a) Experiment 1	A	R	N	D	C	Q	G	H	I	L	K	M	F	P	S	T	Y	V
Cycle	Ala	Arg	Asn	Asp	Glu	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val
1	172.6	0.0	31.9	35.7	44.8	125.9	112.4	2.8	144.4	123.8	60.0	30.7	63.3	117.9	75.9	49.8	50.3	104.9
2	47.5	0.0	16.2	14.1	25.6	53.1	44.7	3.6	69.6	511.0	15.5	71.0	10.5	30.7	16.2	16.1	12.2	86.5
3	99.0	0.0	9.5	10.3	12.3	20.4	31.8	11.1	51.5	110.8	5.8	55.7	19.4	30.4	12.0	8.7	20.9	46.0
4	36.0	0.6	12.7	26.4	59.5	21.7	56.2	1.3	10.4	72.7	24.6	5.2	5.2	52.4	10.9	14.0	5.2	70.8
5	35.1	0.1	13.4	10.6	20.1	19.8	55.6	2.8	21.4	23.9	47.2	4.1	6.2	39.1	7.5	10.5	11.6	29.0
6	30.3	0.0	16.8	14.1	21.4	17.3	20.5	1.4	60.1	43.4	14.7	4.4	5.8	40.8	8.2	20.3	5.0	106.2
7	47.1	0.0	11.7	9.5	27.2	21.0	19.0	3.2	36.3	27.3	7.9	5.7	8.0	54.1	5.4	13.6	14.0	62.8
8	37.9	0.3	13.4	8.1	37.3	24.3	21.1	1.8	11.6	15.1	33.8	3.4	5.1	22.3	8.8	17.9	10.2	22.4
9	23.3	0.0	5.1	6.0	15.7	10.5	14.8	0.7	11.5	27.5	8.7	3.1	2.7	11.9	5.6	6.7	5.1	60.7
10	12.0	0.7	2.6	4.4	6.5	5.2	10.2	0.4	4.5	12.1	4.5	1.0	1.8	7.1	2.7	3.2	2.3	20.4
(b) Experiment 2																		
1	110.8	10.8	4.0	3.1	10.0	14.5	55.7	0.2	60.3	44.4	10.8	8.2	37.5	20.3	27.1	14.8	19.8	48.0
2	13.4	1.6	2.0	1.9	6.8	11.0	9.0	0.0	37.9	302.7	0.0	26.2	5.0	6.3	4.4	4.5	3.3	26.5
3	62.4	3.5	5.0	9.1	4.9	10.0	12.6	0.1	35.7	71.5	0.0	24.5	13.0	13.4	8.9	4.8	12.9	19.6
4	16.9	2.3	4.5	8.8	25.3	7.9	24.5	0.1	6.2	10.3	2.8	1.3	2.0	22.1	4.9	5.0	1.8	9.3
5	22.3	1.6	6.8	6.6	14.3	9.9	31.8	0.0	16.6	15.1	8.2	1.9	4.0	16.3	4.5	4.6	5.7	10.3
6	10.6	1.3	6.6	3.6	6.4	6.2	10.1	0.1	30.7	27.1	0.0	1.4	2.7	12.6	3.2	6.1	1.3	39.2
7	19.2	1.0	4.7	2.5	7.2	9.0	5.6	0.2	22.3	16.1	0.0	1.9	3.9	17.4	1.9	3.5	3.6	27.3
8	13.4	1.2	3.1	1.3	7.9	6.3	6.9	0.3	4.7	6.7	3.0	0.6	2.0	5.1	2.2	4.9	1.6	5.3
9	5.7	0.5	0.9	0.8	2.9	2.0	2.7	0.2	3.8	11.5	0.4	0.3	0.6	2.0	1.0	1.1	0.4	10.8
10	2.9	0.6	0.5	0.5	1.0	0.9	1.8	0.3	1.6	5.1	0.4	0.3	0.3	0.8	0.4	0.3	0.2	3.6

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Table 4c

The HLA-A2.1-restricted peptide motif (HLA-A*0201)

	Position								
	1	2	3	4	5	6	7	8	9
Dominant anchor residues		L							V
strong		M		E K		V		K	
weak	I		A	G	I	I	A	E	L
	L		Y	P	K	L	Y	S	
	F		F	D	Y	T	H		
	K		P	T	N				
	M		M		G				
	Y		S		F				
	V		R		V	H			

Known epitopes

	Protein source	Literature reference
I L K E P V H G V	HIV reverse transcriptase 461-485	43
G I L G F V F T L	influenza matrix protein 57-68	44
I L G F V F T L T V	influenza matrix protein 57-68	44
F L Q S R P E P T	HIV Gag protein 446-460	46
A M Q M L K E . .	HIV Gag protein 193-203	46
P I A P G Q M R E	HIV Gag protein 219-233	46
Q M K D C T E R Q	HIV Gag protein 418-443	46

Table 5
The HLA-A*0205-restricted peptide motif

	Position								
a) A*0205	1	2	3	4	5	6	7	8	9
Dominant anchor residues									L
others		V	Y	G	V	I	Q	K	
		L	P	E	Y	V			
		I	F	D	L	T			
		Q	I	K	I	L			
		M		N		A			
						R			

Table 6
The H-2K^k-restricted peptide motif

	Position							
	1	2	3	4	5	6	7	8
Dominant anchor residues		E						I
strong			K					
			N					
			Y					
			M					
weak	V		Q	L	A	H	T	
	F		I		G	K		
			L		P	H		
			F		T			
			P		V			
			H		F			
			T		S			

Table 7
The H-2K^{bm1}-restricted peptide motif

	Position							
	1	2	3	4	5	6	7	8
Dominant anchor residues							I	
strong		E	K					
weak		Q	N	T	A		R	
		G	Q		R		Y	
		P	G		K			
			M					
			P					
			Y					

Example 4

Peptide motifs of HLA-A1, HLA-A3, HLA-A11, HLA-A24, HLA-A31, HAL-A33, HLA-B7, HLA-B8, HLA-B*2702, HLA-B*3501, HLA-B*3503, HLA-B37, HLA-B38, HLA-B*3901, HLA-B*3902, HLA-B*5101, HLA-B*5102, HLA-B*5103, HLA-B*5201, HLA-B58, HLA-B60, HLA-B61, HLA-B62, HLA-B78, HLA-Cw*0301, HLA-Cw*0401, HLA-Cw*0502, HLA-Cw*0702, HLA-Cw4, HLA-Cw6, HLA-Cw7, HLA-DRB1*0101, DRB1*-1201, HLA-DR4w14, HLA-DR17, HLA-DRw52, HLA-DPw2, HLA-DPB1*0401, HLA-DQB1*0301, HLA-DQw1, HLA-DR1, HLA-DR3 and HLA-DR5.

The determination of these peptide motifs was carried out according to examples 1 to 3. The results are shown in tables 8 to 24.

The peptide motifs HLA-A, B and C are MHC class I ligands. The peptide motifs HLA-DR, DQ and DP are MHC class II ligands.

Comments on the epitope prediction for HLA class I ligands

Anchors: All anchors are usually used in all natural MHC class I ligands. However, it is also possible that other anchor residues are used than those stated i.e. those having similar properties (e.g. a hydrophobic amino acid may be substituted by another hydrophobic amino acid). The number of amino acids between the anchors is generally constant, however, one or two further amino acids may be inserted.

Auxiliary anchors: These are preferably used but are not obligatory; if several auxiliary anchors are stated then usually at least some of them are used.

For the epitope prediction with regard to a protein sequence it is expedient to proceed as follows:

The protein sequence is screened for anchor residues with the correct spacing. The partial sequences found in this way (ligand candidates) are examined for the presence of auxiliary anchor residues at the correct position, which reduces the number of ligand candidates. Those candidates are selected from the remaining candidates which additionally contain further preferred amino acid residues.

Comments on the epitope prediction for HLA class II ligands

Anchors: The HLA class II motifs have 3 or 4 anchors. However, the individual ligands often only use 2 of these anchors. Presumably the ligands which bind particularly strongly utilize all anchors and missing anchor residues can probably be compensated by correspondence with regard to other preferred residues.

In order to illustrate this, the matching anchor residues are double underlined in the ligand examples and the other preferred residues are underlined simply.

The allele-specific motifs in Tables 39 - 47 are stated in terms of relative positions (first anchor = relative position 1) since the number of amino acid resifues between the N-terminus and the first anchor is variable in the case of class II ligands (compared to class I ligands). In Tables 48 - 50 the motifs are given as absolute positions.

The procedure for epitope prediction within a protein sequence is similar to that of class I except that from the start the sequence is examined for correspondence with at least 2 anchor residues (one of which is anchor 1). If several ligand candidates are obtained in this way, the other preferred residues are compared for further limitation and finally tested for correspondence with the (non-allele-specific) processing motif (protein from absolute position 2 and 12 to 16).

A very strong Pro signal is found at the absolute position 2 of the examined HLA class II ligands. Further

Pro signals are present in the region of the C terminus. These Pro signals seem to be a preferred feature of natural class II ligands. The processing motif for HLA class II ligands is thus as follows:

absolute position:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	P										p	p	p	p	p	

In Tables 39 - 47 the first anchor is generally in the region of the absolute positions 3 - 5 or 4 - 6.

The upper part of Figure 2 shows a simplified comparison of class II and class I ligands. Class II ligands (left) are anchored in the MHC cleft by allele-specific pockets. Both ends of the cleft are open i.e. the ligands can protrude (12 - 25 residues, 15 - 16 residues on average). The second residue after the N-terminus is often Pro, probably the result of an aminopeptidase activity. As can be seen from Figure 2, this Pro residue is not involved in binding to the MHC cleft. i.e. a synthetic, MHC II-binding peptide motif does not necessarily have to contain the Pro residue but it preferably starts with the first anchor residue. The distance between the N-termini and the first anchor is 5 ± 1 residues for the majority of ligands. The distance between the last anchor and the C-terminus is not constant. The major differences to ligands of class I (right) are the firm binding of the peptide termini within the cleft and the more defined length of the ligands.

The lower part of Figure 2 shows the hypothetical binding of class II ligands to their receptor. The

ligand is shown as a peptide backbone in an extended orientation. The first hydrophobic anchor is at the α-end of the cleft and the last is at the opposite end. The second anchor is approximately in the middle where the α and β domains meet. Thus the distance between the first and the last anchor corresponds to the length of the cleft. The relatively conservative characteristics of the first anchor (hydrophobic/aromatic) of the various alleles can mirror the absence of an increased polymorphism in the DNA genes whereas the second and the last anchor are subjected to the influence of polymorphous β chains.

Table 8: HLA-A1 motif

	Position 1	2	3	4	5	6	7	8	9
Anchor residues									
		T	D				L		Y
or auxiliary anchor residues		S	E						
other preferred residues		L		P	G	G			
				G	N	V			
				I	Y	I			
Examples of ligands		A	T	D	F	K	F	A	M	Y
		I	A	D	M	G	H	L	K	Y
		M	I	E	P	R	T	L	Q	Y
		Y	T	S	D	Y	F	I	S	Y
		L	T	D	P	G	V	L	D	Y

Table 9: HLA-A3 motif

	Position 1	2	3	4	5	6	7	8	9	10
Anchor residues										
		L				I	I		K	K
or auxiliary anchor residues		V				M	L		Y	
		M				F	M		F	
						V	F			
						T				
						L				
other preferred residues		I		F		I			Q	
				Y		P			S	
						V			T	
						K			K	

Table 10: HLA-A11 motif

	Position 1	2	3	4	5	6	7	8	9	10	11
Anchor residues and auxiliary anchor residues	V	M							K	K	K
	I	L									
	F	F									
	Y	Y									
	T	I									
		A									
other preferred residues	A	N	P	P	I	L	R	R	R	R	
		D	G	I	V	I	K	D			
		E	D	F	M	Y	N				
		Q	E	V		V	E				
			K	M		F	Q				
Examples of ligands	A	V	M	K	P	E	A	E	K	R	K
	A	V	I	L	P	P	L	S	P	Y	F	K

Table 11: HLA-A24 motif

	Position 1	2	3	4	5	6	7	8	9
Anchor residues or auxiliary anchor residues	Y				I	F			I
					V				L
									F
other preferred residues		N	D				Q	E	
		E	P				N	K	
		L							
		M							
		P							
		G							

Table 12:

HLA-A31 motif

	Position								
	1	2	3	4	5	6	7	8	9
Anchor residues or auxiliary anchor residues	L					L			R
	V					F			
	Y					V			
	F					I			
						T			
other preferred residues	K	T	K	P	P	N	N	L	
		Q	N	D	I	D	V	R	
			F	E	V	E	R	N	
			L	G	F	R	F	Q	
			Y	S	L		T		
			W	V	Y		H		
				T	W		L		
							Y		
Examples of ligands	L	Q	F	P	V	G	R	V	H	R
	Q	Q	L	Y	W	S	H	P	R	
	R	G	Y	R	P	R	F	R	R	
	K	V	F	G	P	I	H	E	R	
	K	I	M	K	W	N	Y	E	R	

Table 13:

	HLA-A33 motif								
	Position								
	1	2	3	4	5	6	7	8	9
Anchor residues or auxiliary anchor residues	A								R
	I								
	L								
	F								
	Y								
	V								
particularly preferred residues	T	L	P	P	I				
		K			L				
					F				
other preferred residues	E	Q	R	R	R	H	Q		
	M	W	D	I	D	Y	N		
		E	E	F	H	V	E		
		N	G	P	Y	T	M		
			C	V		S			
			H	L					
			P	W					
Examples of ligands	D	M	A	A	Q	I	T	Q	R
	E	S	G	P	S	I	V	H	R
	E	Y	Y	G	S	F	V	T	R
	D	Y	I	H	I	R	I	Q	Q R
	E	I	M	K	W	N	R	E	R
	E	V	L	D	I	F	Q	D	R

Table 14:

	HLA-B7 motif								
	Position								
	1	2	3	4	5	6	7	8	9
Anchor residues		P							L
									F
other preferred residues	A		D	D	D	F	L		
	H		E	E	P	T	V		
	S		Q	G	I	R			
			K	H	V	L			
			Y	L		I			
			F	K					
			M	S					
			N	T					
			A	P					

Table 15:

HLA-B8 motif

	Position								
	1	2	3	4	5	6	7	8	9
Anchor residues			K		K				L
					R				
particularly preferred residues			G		E		N	E	E
			L		Q		Q	H	Q
			I				H	M	H
							I		S
							L		
							Y		
							V		
other preferred residues					D		E	N	L I
					H		M	D	V
					L		S	Q	D
					S		T	S	T
					T		F	T	
					R			Y	
					G				
					K				

Table 16:

HLA-B*2 J2 motif

	Position								
	1	2	3	4	5	6	7	8	9
Anchor residues									
		R							F
									Y
									I
									L
									W
other preferred residues	K		F	G	I	I	Y	K	
			L	P	K	V	L	V	
			X	K	E	Y	V	D	
				D	V	R	T	E	
				E	M	D	F	R	
				Q	T	H			
				T		E			
				S		Q			
Examples of ligands	S	R	D	K	T	I	I	M	W
	G	R	L	T	K	H	T	K	F
	R	R	F	V	N	V	V	P	T F
	K	R	Y	K	S	I	V	K	Y
	K	R	K	K	A	Y	A	D	F
	K	R	G	I	L	T	L	K	Y
	G	R	F	G	V	G	N	R	Y
	G	R	F	K	L	I	V	L	Y

Table 17:

HLA-B*3501 motif

	position									
	1	2	3	4	5	6	7	8	9	10
Anchor residues or auxiliary anchor residues		P							Y	Y
									F	F
									M	M
									L	L
									I	I
other preferred residues	M	A	I	K	D	I	V	E		
		V	L	D	I	Q	N	Q		
		Y	F	E	V	K	E	V		
		R	V	G	T	V	Q	T		
		D	M	P	E	L	T			
			E		G	M	K			
			T		L					
			Y		M					
			N							

Table 18:

HLA-B*3503 motif

	Position								
	1	2	3	4	5	6	7	8	9
Anchor residues or auxiliary anchor residues		P							L
									M
other preferred residues		A	I	E	G	D	Q	Q	F
		D	L	K	V	E	N	R	
			M	N	H	V	T		
			V	H	I				
			R						

Table 19:

HLA-B37 motif

	Position								
	1	2	3	4	5	6	7	8	9
Anchor residues or auxiliary anchor residues		D			V			F	I
		E			I			M	L
					A			L	
					M				
other preferred residues	K	E			T		Q	T	
	Q	P			R		K	E	
		G			D		Y	N	
		S			G		L	D	
		L			H			Q	
								G	
								H	

Table 20:

	HLA-B38 motif								
	Position								
	1	2	3	4	5	6	7	8	9
Anchor residues									
									F
									L
other									
preferred residues	I	H	I	G	M	V	Y	K	I
		F	A	E	T	I	V	Y	
		P	D	P	V	T	N	N	
		W	E	L	A	K		R	
		Y	S	V	E	R		T	
			N		G	N			
			M		L	H			
			V		K				
					S				
Examples of ligands	E	H	A	G	V	I	S	V	L
	T	H	D	E	L	E	D	K	L
	Q	Y	D	E	A	V	A	Q	F
	Y	P	D	P	A	N	G	K	F

Table 21:

HLA-B*3901 motif

	Position								
	1	2	3	4	5	6	7	8	9
Anchor residues or auxiliary anchor residues	R					I			L
	H					V			
						L			
other preferred residues		A	D	V	N	N	S		
		D	E	Y		Y	K		
		I	G	I		F	R		
		L	P	L			E		
		F	K	F			T		
		V		T					
		M		G					
		S		K					
		T		N					
		Y		P					

Table 22:

HLA-B*3902 motif

	Position								
	1	2	3	4	5	6	7	8	9
Anchor residues or auxiliary anchor residues		K			I				L
		Q			L				
					F				
					V				
other preferred residues	K		A	G	N	V	V	T	F
	A		I	P	E	Y	L	S	M
			F		G	T	T	R	
			V		P	H	Y		
			N		Q	F	N		
			L		S	I	D		
			T		T	M	H		
			Y			P			
			E			R			
			H						
			S						

Table 23:

	HLA-B*5101 motif								
	Position								
	1	2	3	4	5	6	7	8	9
Anchor residues or auxiliary anchor residues		A							F
		P							I
		G							
other preferred residues	I	W	I	G	V	N	K	T	W
	L	F	L	V	T	I	Q		M
	V		M	I	G	L	R		V
	Y		F	K	A	K	E		
			W	E	I	Q			
			Y	D	S				
			V						
			E						
			H						
			D						
			R						
			N						
Examples of ligands	Y	P	F	K	P	P	K	V	
	D	A	H	I	Y	L	N	H	I
	T	G	Y	L	N	T	V	T	V
	X	A	Y	A	L	N	H	T	L

Table 24:

HLA-B*5102 motif

	Position								
	1	2	3	4	5	6	7	8	9
Anchor residues or auxiliary anchor residues	P	Y							I
	A								V
	G								
other preferred residues		F	C	V	I	R	T		
		V	E	Q	N	Ξ	R		
		L	K	N	Q	Q	Y		
		I	L	G	T	K			
			T	T					
			Q						
			R						
			N						
			H						

Table 25:

HLA-B*5103 motif

	Position						
	1	2	3	4	5	6	7
Anchor residues or auxiliary anchor residues	A	Y					V
	P						M
	G						
other preferred residues	F	F	E	G	I		
	W	D	L	A	K		
		L	N	V	T		
			R	N			
			G	Q			
			Q	M			
			T	R			
			V				

Table 26:

	HLA-B*5201 motif							
	Position							
	1	2	3	4	5	6	7	8
Anchor residues or auxiliary anchor residues		Q	F		L			I
			Y		I			V
			W		V			
other preferred residues	V	M	I	L	M	K	K	
	L	F	L	I	F	N	E	
	I	P	P	V	A	L	Q	
			D	P	T	T	Y	
			K	K	G	S		
				E				
				A				
Examples of ligands	T	G	Y	L	N	T	V	T V
	V	Q	T	I	M	P	Q	L

Table 27:

	HLA-B58 motif								
	Position								
	1	2	3	4	5	6	7	8	9
Anchor residues or auxiliary anchor residues	A			P	V				F
	S			E	I				W
	T			K	L				
					M				
					F				
other preferred residues	K	G	G	D	A	I	L	N	Y
	R		T	Q	D	V	Y	R	
	I		I	R	N	L	M	K	
			L		T	F	N	T	
			V			Y			
			F			W			
			Y			Q			
			N						
			Q						
Examples of ligands	K	A	G	Q	V	V	T	I	W
	A	G	D	R	T	F	Q	K	W

Table 28:

	HLA-B60 motif								
	Position								
	1	2	3	4	5	6	7	8	9
Anchor residues or auxiliary anchor residues		E					I		L
							V		
other preferred residues		A	P	L	K	L	K		
		V	K	I	N	Y	R		
		I	D	V	P	M	Q		
		L	G	D	V				
		M	N	T	I				
		F	Q	N	D				
		S	T	P	R				
		D		G	Q				
		N		X					
				Q					
Examples of ligands		K	E	S	T	K	H	L	
		H	E	A	T	L	R		
		Y	E	I	H	D	G	M	N	L

Table 29:

	HLA-B61 motif							
	Position							
	1	2	3	4	5	6	7	8
Auxiliary anchor residues								
		E	F			I		
			I			L		
			L			F		
			V			V		
			Y					
			W					
other preferred residues	P	M	E	V	W	Y	K	A
		T	G	I		V	S	P
			P	L		L		
			S	M		W		
			N	D		I		
			D	G		T		
			K	V		R		
			A	F		D		
			R	N		Q		
			N	S		G		
			Q	K				
Examples of ligands	G	E	F	G	G	F	G	S	V
	E	E	F	Q	F	I	K	K	A
	G	E	F	V	D	L	Y	V	

Table 30:

	HLA-B62 motif
	Position
	1 2 3 4 5 6 7 8 9
Anchor residues or auxiliary anchor residues	Q I F
	L V Y
other preferred residues	I M K P G V V Y
	V A E L T T V
	N G F G L T
	F D T I I
	P
	Y
	H
	R
Examples of ligands	V L K P G M V V T F
	Y L G E F S I T Y

Table 31:

HLA-B78 motif

	Position							
	1	2	3	4	5	6	7	8
Anchor residues or auxiliary anchor residues	P					I		A
	A					L		
	G					F		
						V		
other preferred residues		Y	E	D			A	K
		D	D	G			V	S
		W	G	V			N	
			L	N			K	
			V	R			Q	
			S	Q			E	
			Q	S				
			R	T				
			N					

Table 32:

Cw*0301 motif

	Position								
	1	2	3	4	5	6	7	8	9
Anchor residues or auxiliary anchor residues		V	P			F			L
		I				Y			F
		Y							M
		L							I
		M							
other preferred residues	R	E	R	N	M	Q	T		
	N					K			
						S			
						M			

Table 33:

HLA-Cw*0401 motif

	Position								
	1	2	3	4	5	6	7	8	9
Anchor residues or auxiliary anchor residues	Y					V			L
	F					I			F
						L			M
other preferred residues	P	D	D	A	X			K	
		H	E	H			A	S	
			P	M			X	H	
			X	T				K	
				R					

Table 34:

	HLA-Cw*0602 motif								
	Position								
	1	2	3	4	5	6	7	8	9
Anchor residues or auxiliary anchor residues					I	V			L
					L	I			I
					F	L			V
					M				Y
other preferred residues	I	P	P	P	K	A	R		Y
	F	R	I	E		T	K		E
	K		G	D		S	Q		Q
	Y		F	Q			N		N
			Y	L					R
			K						G
			N						T
			A						S
									K
Examples of ligands	Y	Q	F	T	C	I	K	K	Y
	V	R	H	D	G	G	N	V	L
	F	A	F	P	L	I	Q	R	V
	X	Q	R	T	P	K	A	G	L	Y	Y

Table 35:

	HLA-Cw*0702 motif								
	Position								
	1	2	3	4	5	6	7	8	9
Anchor residues or auxiliary anchor residues	Y				V	V			Y
					Y	I			F
					I	L			L
					L	M			
					F				
					M				
other preferred residues	R	P	D	D	T	A	Y		E
	D	G	E			R	M		A
		A	V				N		F
			Q				R		D
			P				V		K
			S				F		
			G				Y		
Examples of ligands	K	Y	F	D	E	H	Y	E	Y
	R	Y	R	P	G	T	V	A	L
	N	K	A	D	V	I	L	K	Y
	I	Y	P	Q	N	V	I	L	Y
	I	R	K	P	Y	I	W	E	Y
	N	Y	G	G	G	N	Y	G	S G S Y
	F	Y	P	P	Y	L	Y		

Table 36:

HLA-Cw4 motif

	Position								
	1	2	3	4	5	6	7	8	9
Anchor		F							L
		Y							M
									F
strong	F	P	D	E	A	V	A	H	
	Q				M	I	N	K	
						L	E		
							H		
weak		N	N	R	F	S	Q	I	
		E	R	T	H	Q	S		
		G	D	K	D	D			
			P	F	r	r			
			K	H	S	G			
			Q	M	E	T			
			G		K	Y			
			H		P				
			L		S				
			S						
			T						

Table 37:

	HLA-Cw6 motif								
	Position								
	1	2	3	4	5	6	7	8	9
Anchor									L
									I
									M
									V
strong			P	D	I	V	R	K	
			I	E	M	I	N	F	
			F	P	F		Q	Y	
			Y	N				E	
			N						
			D						
weak	I	P	G	G	L	A	Y	S	
	r	R		V	T	K			
		K		T					
				G					

Table 38:

	HLA-Cw7 motif								
	Position								
	1	2	3	4	5	6	7	8	9
Anchor		Y							L
									F
									Y
									m
strong		P	D	Y		Y			
		F	E	K		I			
			P			V			
weak	p	N		I	T	M	A		
	r	G		F	A	F	E		
		R		V		Y	k		
				A		V	s		
				M		D			

According to this HLA, Cw4, Cw6 and Cw7 ligands have the following properties:

1.) Usually a length of 9 amino acids (longer and shorter peptides can occur)
2.) Mainly aromatic residues F or Y at position 2
3.) Mainly hydrophobic residues V, I, L, F, A at position 6 ("auxiliary anchor")
4.) Hydrophobic residues L, F, Y, M, V at the C-terminus.

Individual differences in the ligand specificity of Cw4, Cw6 and Cw7 are shown by the tables.

Table 39: HLA-DRB1*0101 motif

	Position										
	-1	0	1	2	3	4	5	6	7	8	9	10
Anchor residues			Y			L					L	
			V			A					A	
			L			I					I	
			F			V					V	
			I			M					M	
			A			N					F	
			M								Y	
			W									
preferred residues												
polar or charged	K		K	K		E		H	H		K	
	Q		D	D		Q		R	R		R	
	E		E	E		D		D	D		Q	
	N		R	R		H		Q	Q		D	
	D		H	H		R						
	R											
	H											
preferred small residues			A	A		S	A	S	S			
			T	S		T	G	T	T			
					P	P	S	P				
							T					
							P					

Table 40:

HLA-DRB1*1201 motif

	Relative Position										
	-1	0	1	2	3	4	5	6	7	8	9	10
Anchor residues			I	L			V		Y			
		L	M		Y			F				
		F	N		F			M				
		Y	V		I			I				
		V	A		N			V				
					A							
preferred residues polar or charged		N		K			K			R		D
		K		Q			E			K		R
		E		E			Q			N		H
		D		R			R			Q		E
				H			H			D		K
				D								
preferred small residues				A		G	A	A		A		A
				T		P	G	G		G		G
							S	T		S		S
							T	P				T
							P		S			
									P			
Examples of ligands			S	S	V	I	T	L	N	T	N	V	G	L	Y	X	Q	T
						I	K	L	L	N	E	N	S	Y	V	P		
		G	P	D	G	R	L	L	R	G	Y	D	Q	F	A	Y	D	G	K
			S	D	E	K	I	R	M	N	R	V	V	R	N	N	L	R	
		I	N	Q	K	G	L	S	G	L	Q	P	L	R	F	L		
			E	A	L	I	H	Q	L	K	I	N	P	Y	V	L	S		

Table 41:

HLA-DR4w14 motif

	Relative position										
	-1	0	1	2	3	4	5	6	7	8	9	10
Anchor residues												
		I			F			I				
		L			Y			V				
		M			I			L				
		V			V			M				
		Y			L			Y				
		F			M			A				
preferred residues, polar or charged		H			K		Q	D	D			
		Q			R		N	H	H			
		N			Q		E	Q	Q			
		E			N		D	N	N			
		D			H							
		Q										
		R										
preferred small residues						A		G	T	A		
								T	S			

Examples of ligands

	G	S	A	S	M	R	Y	F	H	T	A	M	S	R	P	G	R	G	E	F
V	D	D	T	Q	F	V	B	F	D	S	D	A	A	S	Q	R	M	E	P	
	Y	D	N	S	L	K	I	I	S	N	A	S	X	T	T	N				

Table 42: HLA-DR17 motif

	Position																
	-1	0	1	2	3	4	5	6	7	8	9	10	11				
Anchor residues or auxiliary anchor residues			L I F Y M V			D		X R E Q N		L	Y L F	F Y	F				
other preferred residues		N	Q		T	K P Y N		K	E Q R			A			D		
Examples of ligands	I	S V P K Y N L N	N D R Q P I L X	Q T A T D Q S F	L F I I F L F L	T L V S I I V X	L E V P M H R S	D D D D D D D D	S V P Y P Q L G	N K V R E E N R	T N H N E V Q V	K L G M K A Y K	Y Y F I D R R X	F A M K E A T	H S X V D D L	K D I N	L A
	I X D																

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Table 43:

HLA-DRw52 motif

	Relative Position										
	-1	0	1	2	3	4	5	6	7	8	9	10
Anchor residues			F			N			L			
			I			L			Y			
			L			V			I			
			Y			I			V			
			M			Y						
			A			A						
other preferred residues		E	A		A	A		A	T			E
		K	T					E	G			K
		Q						R	K			Q
		N										

Examples of ligands

		S	L	Q	F	G	Y	N	T	G	V	I	N	A P Q
S	S	V	I	I	L	N	T	N	V	G	L	Y	X	Q S
			N	F	E	R	N	K	A	I	K	V	I	
		V	T	R	Y	I	Y	N	R	E	E	Y	A	R F
	V	V	A	P	F	M	A	N	I	P	L	L	L	Y

Table 44:

HLA-DPw2 motif

	relative position									
	-1	0	1	2	3	4	5	6	7	8	9
Anchor residues			F				F			I	
			L				L			A	
			M				M			M	
			V				Y			V	
			W								
			Y								
preferred residues, polar or charged		R			D		N			N	
		N			H		D			Q	
		Q			Q		H			H	
		H			R		K			R	
		K									
		E									
preferred small residues						S				S	
						A				T	
						T				A	

Examples of ligands

L F R K F H Y L P F L P S T E D V

L P R E D H L F R K F H Y L P F L P S

V T N K F P T Q L F H T I G V E

A D E K K F W G K Y L Y E I A R R H P

D S F K L Q T K E F Q V L K S L G

G E P L S Y T R F S L A R Q V D G

Table 45:

HLA-DQB1*0301 motif

	Relative Position												
	-1	0	1	2	3	4	5	6	7	8	9	10	11
Anchor residues			F Y I M L				V L I M Y		Y F M L V I				
preferred residues polar or charged	D K E N Q			Q H N K R				N H Q E K				H D Q N K R	
preferred small residues		A G T S P		A G T S	A G T S	A	S P			G T S P	T	T	
Examples of ligands	D S	T V K	B E M	L V R	L Y M	M B A	Q A T	A V P	L T L	P P L	M L M	G G Q	A P A	L	P	Q
	K	P	P	K	P	U										

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Table 46:

HLA-DPB1*0401 motif

	Relative Position										
	-1	0	1	2	3	4	5	6	7	8	9	10
Anchor residues												
			F						F		V	
			L						L		Y	
			Y						Y		I	
			M						M		A	
			I						V		L	
			V						I			
			A						A			
preferred residues, polar or charged		K					N					
		R					K					
		E					E					
		N										
		Q										
preferred small residues					A							
					V							
Examples of ligands	X	K	K	Y	F	A	A	T	Q	F	E	P	Y	N	N
	G	P	G	A	P	A	D	V	Q	Y	D	L	Y	L	N	V	A	N	R	R

Table 47:

HLA-DQw1 motif

	Relative Position									
	-1	0	1	2	3	4	5	6	7	8	9
Anchor residues										
			L		F			L			
			F		Y			I			
			N		V			W			
								V			
other preferred residues		E		A			P				
		R		E							
		T		G							
				H							
				N							
				Q							
				R							
				S							
				T							

D T L R S Y Y A D W Y Q Q K P G
F K T L D I D R F E P L

Table 48a HLA-DR1 motif

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
very strong		P																		
strong		E		D	F	R	L	R	N		M	T								
				F	T	H	M	N	P		D									
					Y	I	S	G			P									
						K		K												
								V												
weak	G	D	P	Q	D	N	a	S	M	Q	R	A	K	F	E					
		G	t	Y	G	M	q	T	a	M	Q	l	A		Q					
		i	N	A	I	S	v	Q	s	a			I							
		N	I	N	a	a	t	a	l				T							
			L	E	l	g		l					V							
			K	G	v	v														
			M	L	e															
			F	M																
			D	S																
			E																	
			Q																	

Table 48b:: Interpretation: HLA-DR1 motif

1	2	3	4	5	6	7	8	9	10	11	12	13
	P											
E	N	D						M	M	M		
D	K	Q						A	A			
N	D	N						L				
	E	E										
	Q											
	I	F	F	I								
	L	Y	Y	M								
	M	A	I	A								
	F	L	A	V								
		M	L									
			V									

Thus natural DR1 ligands have the following properties:

1.) Length usually more than 11 amino acids (is already known)

2.) Mainly P at the second position

3.) Polar/charged residues E, D, K, N, K, Q at position 2, 3 or 4

4.) Hydrophobic residues I, L, M, F, A at position 3, 4, 5 or 6

5.) Hydrophobic residues M, A or L at position 9, 10 or 11

Table 49a:
HLA-DR3 motif

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
very strong			P													
strong	A		R	D	D	D	R	Q	R	L	L	T	F	F	S	A
	I		Q	F	L	Q	D	K	K	K	E	Y	L	K	T	
	P		L	L	S	K	K	r	S	K	Q	L	Y	y	E	
	V		F	A	t	S	Q	D	L		F	G				
			G	I	y	Y	H	T	Y		A					
			H	M	Q	M		N	N							
			D	T	I	P		E	d							
			I	R	F			G	H							
				E												
				Q												
				G												
				Y												

Table 49b:

Interpretation:

HLA-DR3 motif

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
P															
		L	F	L						L	Y	F	F		
		F	L	I						F	L	L	Y		
		I	I	Y						A		Y			
			Y	F											
			M												
			A												
			D	D	D	D	Q	D							
			R	Q	K	K	K	K							
			E		Q	Q	R	R							
			Q			R	E	E							
						H		N							
								H							

Thus natural DR3 ligands have the following properties:

1.) as for DR1

2.) as for DR1

3.) hydrophobic residues L, F, I, Y, M, A at position 3,4 or 5

4.) polar,charged residues at position 4,5,6,7,8 or 9

5.) hydrophobic residues L,F,A,Y at position 11,12 or 13

Table 50a:

HLA-DR5 motif

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
very strong	P																
strong	N	D	N	R	R	R	R	P	N	R	m	D	A	i			
	g	N	D	I	N	N	N	i	D	E	Q	M	P				
		E	E	L	Q	L	L	v	E	q	Y	Y					
		L	G	F	L	A	D	A	M		r	F					
		T	I	t	K	q		E	Y			V					
		Y	L	Y	A	M		G	V			P					
		H	K	V	H	v		h	p								
		V	M	a	M			k	q								
			F	n				S	a								
			Y	K					r								
			V						H								
			H														
			r														

Table 50b:
Interpretatior:
HLA-DR5 mot:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
P																			
N	D	D	R	R	R	R			N	R									
	E	E	K	N	N	N			D	E									
	N	N	n	Q	q	d			E	Q									
	H	K		K					Q										
		H		H															
L	L	L	L	L	L														
Y	Y	Y	M																
	I	I																	
	M	V																	
	F	F																	
	V																		

Thus natural DR5 ligands have the following properties:

1.) as DR1
2.) as DR1
3.) polar/mainly negatively charged residues N, D, E, H, K at position 2, 3, or 4
4.) polar/mainly positively charged residues R, K, N, Q at position 5, 6, 7 or 8
5.) hydrophobic residues L, Y, V, I, M, F, A at position 3, 4 or 5 as well as 5, 6 or 7
6.) polar/charged residues N, D, E, H, R, Q at positions 10, 11 or 12

Literature references

1. Zinkernagel, R.M. & Doherty, P.C., Nature 248, 701-702 (1974).
2. Townsend, A.R. et al., Cell 44, 959-968 (1986).
3. Bjorkman, P.J. et al., Nature 329, 512-518 (1987).
4. Rötzschke, O. et al., Nature 348, 252-254 (1990).
5. VanBleck, G.M. & Nathenson, S.G., Nature 348, 213-216 (1990).
6. Garrett, T.P.J., Saper, M.A., Bjorkman, P.J., Strominger, J.L. & Wiley, D.C., Nature 343, 692-696 (1989).
7. Rötzschke, O., Falk, K., Wallny, H.-J., Faath, S. & Rammensee, H.-G., Science 249, 283-287 (1990).
8. Falk, K., Rötzschke, O. & Rammensee, H.-G., Nature 348, 248-251 (1990).
9. Shimonkevitz, R., Kappler, J., Marrack, P. & Grey H., J.exp.Med. 158, 303-316 (1983).
10. Demotz, S., Grey, H.M., Appella, E. & Sette, A., Nature 343, 682-684 (1989).
11. Bjorkman, P.J. et al., Nature 329, 506-512 (1987).
12. DeLisi, C. & Berzolsky, J.A., Proc.natn.Acad.Sci.USA 82, 7048-7052 (1985).
13. Rothbard, J.B. & Taylor, W.R., EMBO J. 7, 93-100 (1988).
14. Cornette, J.L., Margaht, H., DeLisi, C. & Berzolsky, J.A., Meth.Enzym 178, 611-633 (1989).
15. Sette, A. et al., Proc.natn.Acad.Sci.USA 86, 3296-3300 (1989).
16. Maryanski, J.L., Verdini, A.S., Weber, P.C., Salemme, F.R. & Corradin, G., Cell 60. 63-72 (1990).
17. Bastin, J., Rothbard, J. Davey, J. Jones, I. & Townsend, A., J.exp.Med. 165, 1508-1523 (1987).
18. Bjorkman, P.J. & Davis, M.M., Cold Spring Harb.Symp. quant.Biol. 54, 365-374 (1989).
19. Boulliot, M. et al., Nature 339, 473-475 (1989).
20. Frelinger, J.A., Gotch, F.M., Zweerink, H., Wain, E. & McMichael, A.J., J.exp.Med. 172, 827-834 (1990).
21. Schild, H., Rötzschke, O., Kalbacher, H. & Rammensee, H.-G., Science 247, 1587-1589 (1990).
22. Townsend, A. et al., Nature 340, 443-448 (1989).
23. Elliott, T., Townsend, A. & Cerundolo, V., Nature 345, 195-197 (1990).
24. Cerundolo, V. et al., Nature 345, 449-452 (1990).
25. Rüsch, E., Kuon, W. & Hämmerling, G., J.Trans.Proc. 15, 2093-2096 (1983).
26. Lembke, H., Hämmerling, G.J. & Hämmerling U., Immunol.Rev. 47, 175-206 (1979).
27. Ozato, K. & Sachs, D.H., J.Immun. 126, 317-321 (1981).
28. Parham, P. & Brodsky, F.M., Sum.Immun. 3, 277-299 (1981).
29. Taylor, P.M., Davey, J., Howland, K., Rothbard, J.B. & Askonas, B.A., Immunogenetics 26, 267-272 (1987).

30. Braciale, T.J. et al., J.exp.Med. 166, 678-692 (1987).
31. Braciale, T.J., Sweetser, M.T., Morrison, L.A., Kittlesen, D.J. & Braciale, V.L., Proc.natn.Acad.Sci.USA 86, 277-281 (1989).
32. Kuwano, K., Braciale, T.J. & Ennis, F.A., FASEB J. 2, 2221 (1988).
33. Maryanski, J.L., Pala, P., Cerottini, J.C. & Corradin, G.J., J.Exp.Med. 167, 1391-1405 (1988).
34. Maryanski, J.L., Pala, P., Corradin, G., Jordan, B.R. & Cerottini, J.C., Nature 324; 578-579 (1986).
35. Sibille, C. et al., J.exp.Med. 172, 35-45 (1990).
36. Romero, P. et al., Nature 341, 323-326 (1989).
37. Weiss, W.R. et al., J.exp.Med. 171, 763-773 (1990).
38. Kast, W.M. et al., Cell 59, 603-614 (1989).
39. Oldstone, M.B.A., Whitton, J.L., Lewicki, H. & Tishon, A., J.exp.Med. 168, 559-570 (1988).
40. Tevethia, S.S. et al., J.Virol. 64, 1192-1200 (1990).
41. Carbone, F.R. & Bevan, M.J., J.exp.Med. 169, 603-612 (1989).
42. Schumacher, T.N.M. et al., Cell 62, 563-567 (1990).
43. Walker, B.D. et al., Proc.natn.Acad.Sci.USA 86, 9514-9518 (1989).
44. Gotch, F., McMichael, A. & Rothbard, J., J.exp.Med. 168, 2045-2057 (1988).
45. Santos-Aguado, J., Commins, M.A.V., Mentzer, S.J., Burakoff, S.J. & Strominger, J.L., Proc.natn.Acad.Sci.USA 86, 8936-8940 (1989).
46. Clavene, J.M. et al., Eur.J.Immun. 18, 1547-1553 (1988).
47. Falk, K. et al., J.exp.Med. A4, 425-434 (1991).

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New Claims

1. Use of peptide motifs on molecules of the major histocompatibility complex (MHC) of classes I or II which are selected from the group comprising HLA-A1, HLA-A3, HLA-A11, HLA-A24, HLA-A31, HLA-33, HLA-B7, HLA-B8, HLA-B*2702, HLA-B*3501, HLA-B*3503, HLA-B37, HLA-B38, HLA-B*3901, HLA-B*3902, HLA-B*5101, HLA-B*5102, HLA-B*5103, HLA-B*5201, HLA-B58, HLA-B60, HLA-B61, HLA-B62, HLA-B7[illegible], HLA-Cw*0301, HLA-Cw*0401, HLA-Cw*-0602, HLA-Cw*0702, HLA-Cw4, HLA-Cw6, HLA-Cw7, HLA-DRB1*0101, DRB1*1201, HLA-DR4w14, HLA-DR17, HLA-Drw52, HLA-DPw2, HLA-DPB1*0401, HLA-DQB1*0301 and HLA-DQw1 and which can be obtained by
w h e r e i n
   (a) a cell extract is produced by lysing cells which contain appropriate MHC molecules,
   (b) MHC molecules with the peptide mixtures which are located thereon are separated from the cell extract by immunoprecipitation,
   (c) the peptide mixtures are separated from MHC molecules and other protein components,
   (d) individual peptides or/and a mixture thereof are sequenced and
   (e) the respective allele-specific peptide motif is derived according to one of the tables 8-47 from the informations obtained, in particular

from the sequencing of a mixture or from the sequencing of a number of individual peptides in a process for the production of a diagnostic or therapeutic agent.

2. Use as claimed in claim 1 for the diagnostic detection of MHC molecules.

3. Use as claimed in claim 2,
w h e r e i n
a peptide which corresponds to a peptide motif is coupled with a marker group, in particular a biotin or fluorescent group.

4. Use as claimed in claim 2 for the therapy of disorders of the immune system or of tumour diseases.

5. Use as claimed in claim 4 for the therapy of autoimmune diseases, transplant rejections or/and graft-versus-host reactions.

6. Use as claimed in claim 1 or 5,
w h e r e i n
a peptide which corresponds to a peptide motif is covalently linked N- or/and C-terminally to lipophilic or amphiphilic groups, in particular also lipophilic peptide helices.

7. Use as claimed in claim 6,
w h e r e i n
the lipophilic or amphiphilic group is tripalmitoyl-S-glycerylcysteinyl-seryiserine.

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Fig. 1a

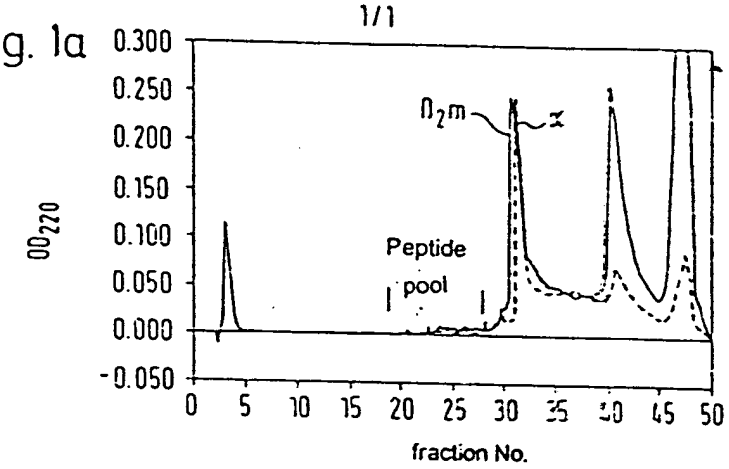

Fig. 1b

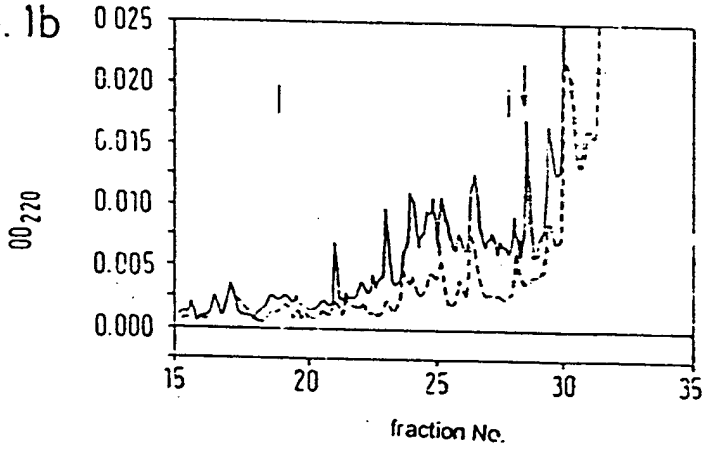

Fig. 1c

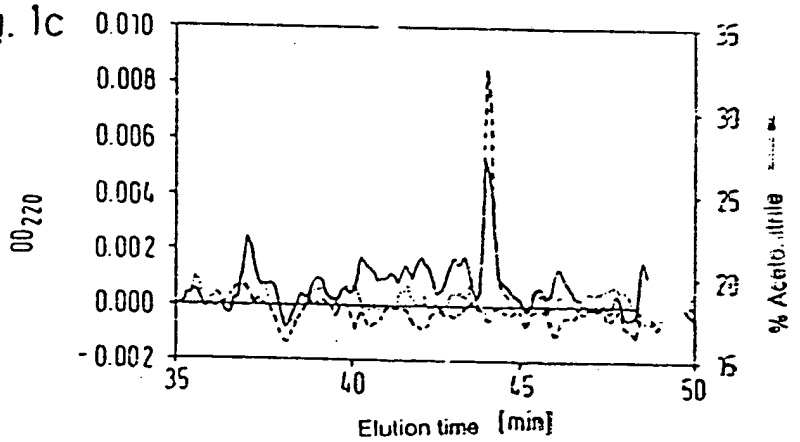
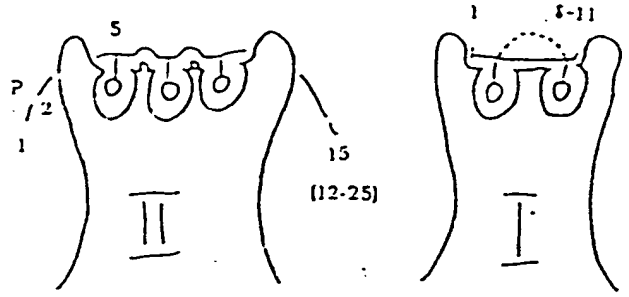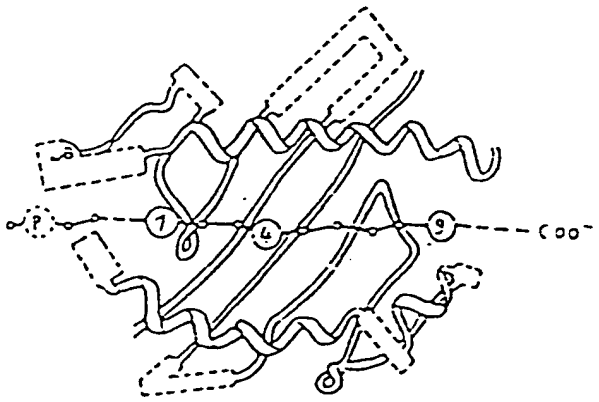
FIG. 2